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LIVE BACTERIAL VACCINES IN THE SOVIET UNION

[Following is the translation of an article by A. T. Pavlenko and R. A. Baltyshev, Central Institute of Medical Biological Preparations imeni L. A. Tarasevicha, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology, and Immunobiology), No 3, 1967, pages 8-9. It was submitted on 17 Oct 1966.]

(Review)

Live Bacterial Vaccines

After the Great October Socialist Revolution, when a prophylactic trend was at the base of the Soviet public health system, specific prophylaxis of infectious diseases naturally became a very urgent problem and received as rapid development as possible.

Already on 10 Apr 1919 the Soviet of People's Commissars of the RSFSR issued a decree, signed by V. I. Lenin, concerning the compulsory small pox vaccination of the entire population of the country.

In the first years of creation of the Soviet public health system one of the main missions was the organization of measures against such social diseases as tuberculosis. In connection with this the attention of Soviet doctors was drawn to the discovery by two French microbiologists Calmette and Guerin of a new method for the active prophylaxis of this disease with the help of the live BCG vaccine. The first experiments at immunization with this vaccine in France were carried out in 1921-1924, and beginning with 1925, after a thorough preliminary study, the new prophylactic preparation was put into use at first on the territory of the Soviet Ukraine, then in Moscow, and later throughout the entire country.

In 1936 the appearance of foreign reports concerning the high degree of effectiveness of live vaccines against plague also aroused the interest of Soviet microbiologists and immunologists, who had obtained local vaccine strains of the plague microbe (Pokrovskaya, 1934, Zhukov-Verezhnikov, 1960; Korobkova, 1956, and others).

In the post-October period the development of new live vaccines and their use in anti-epidemic practice in the Soviet Union were expanded considerably.

Of the number of live bacterial vaccines, created in the USSR

on the basis of the original Soviet vaccine strains and which occupy a secure position among practical measures against infectious diseases of man, it is necessary to note in particular the live anthrax and tularemia vaccines.

The known live vaccines against anthrax (Pasteur, Tsenkovskiy, and others), which are used in veterinary practice in various countries, cannot be used for the immunization of humans due to their high reactogenicity and the possibility of postvaccination complications. In 1940 Ginsburg (1946) selected from a highly virulent anthrax culture a capsule-less variant (mutant) which was designated STI-1 (for the name of the Sanitary-technical Institute in which the author carried out his investigations). Somewhat later in that same laboratory Tamarin isolated another capsule-less variant - No 3. A very comprehensive study of these two strains showed that they hereditarily lost the ability to produce a capsule in the animal organism. This is directly connected with the persistent loss of virulence which is inherent to the anthrax causative agent (Ginsburg, 1946; Saltykov, 1965, and others). On the basis of these two vaccine strains a collective of scientific associates, headed by Ginsburg, developed the technology of production, methods of control, and means of application of the STI live anthrax vaccine.

In 1942-1943 the new STI live anthrax vaccine was put in use for the immunization of farm animals and in more recent years replaced the Tsenkovskiy vaccine (Ginsburg, 1964, 1966). On the basis of experimental data, observed during the mass vaccination of farm animals and experimental inoculation of volunteers in 1944, the STI vaccine was put in limited use for the immunization of people. This was then expanded. Starting with 1950-1951 they started to produce and use this vaccine on a planned scale, primarily for the active prophylaxis of anthrax in persons who were professionally connected with work under threatening conditions (processing of animal raw material of various origin). At the present time a cutaneous (scarification) method has been adopted for a single immunization of people with STI vaccine; as a rule during this general reactions do not develop in those inoculated.

Cases of tularemia began to be recorded in the USSR in 1926. In 1931 research was begun on the active immunization against this disease with vaccines from killed microbes. However, these vaccines, just as analogous preparations in other countries, did not ensure the proper effect. Many years of study on the immunogenesis during tularemia infection led Elbert and Gayskiy (1945) to the conclusion of the necessity for the creation of a live tularemia vaccine. These authors proved the principle feasibility of vaccination not only of animals but also of man with a live culture of a nonpathogenic strain of the tularemia causative agent.

The practical solution of this problem was found after Gayskiy, as a result of purposeful investigations, in 1942 isolated vaccine strains of the tularemia microbe which were harmless for rabbits and guinea pigs and weakly virulent for white mice.

The live tularemia vaccine proposed by Gayskiy (1944) was a suspension of a culture of a vaccine strain in an isotonic solution of sodium chloride. The vaccine was administered subcutaneously one time. The use of the vaccine, at first on volunteers and then in a limited anti-epidemiological experiment, showed the high effectiveness of this vaccine, which, however, had an essential deficiency which is inherent to all other live vaccines: the bacterial cells in the suspension died, which regularly led to a lowering or loss of the activity of the vaccine in 3 weeks after preparation, even when it was stored at a low temperature. This restricted the feasibility of the wide use of the preparation. Connected with the process of dying off of the microbes in the vaccine was the difficulty involved in dosage, and due to error in dosage strong postvaccination reactions were noted during the subcutaneous application of a freshly prepared preparation.

The effectiveness of live tularemia vaccine was raised considerably by the incubation of a vaccine culture in a 10% solution of egg yolk and application of the vaccine not subcutaneously but by the cutaneous (scarification) method (Elbert, Tinner, Puchkova, 1947). The effectiveness of such a method of immunization against tularemia was proven in extensive experiments on animals and by the vaccination of large contingents of people. Reactogenicity of live tularemia vaccine during its cutaneous application was considerably lower. Vaccine which was prepared in yolk medium turned out to be more stable during storage in comparison with that which was suspended in an isotonic solution of sodium chloride, but all the same the period of its suitability during strorage under conditions of low temperature did not exceed 2 months. Moreover a serious deficiency of liquid vitelline vaccine was the impossibility of dosing it out based on the concentration of microbes.

In the USSR the concluding phase in the development of a highly effective and sufficiently stable live tularemia vaccine was the obtaining of a dry preparation. Drying of live vaccine in horse serum was carried out to a limited extent already by Gayskiy together with Ye. A. Golinevich. However, the method used by them could not be reproduced on industrial scales. Development of the method and technology of mass production of live tularemia vaccine was realized by Faybich (1947) and Faybich and Tamarina (1949).

What was mentioned above about the investigations of foreign and Soviet authors demonstrates that the most reliable prophylactic effect is exerted by live vaccines. In experimental investigations and during immunization of the population of regions which are endemic for plague (Madagascar Island) a high degree of effectiveness was displayed by the EV strain (Girard, 1963). However, live plague vaccine, just as other live vaccines made from bacteria which do not form spores, was unstable and non-standard due to the rapid dying off of bacterial cells. This required the organization of production of the preparation at the place where it was to be used and it was not possible to create reserves of vaccine for the timely performance of anti-epidemic measures. In order to eliminate this deficiency of live bacterial vaccines, Faybich and associates at the Scientific-research Institute of Epidemiology and Hygiene used the method of sublimation drying of frozen biological preparations in a deep vacuum, the so-called method of lyophilization. For success in drying there is essential importance in the incubation medium: bacteria which are suspended in distilled water or an isotonic solution of sodium chloride die off rapidly during the process of freezing and drying. After a study of various drying media Faybich and Karneyev (1947) developed disaccharide-colloid media. Of these the widest distribution was given to a dyring medium containing 10% saccharose and 1-1.5% gelatin. They also used modifications of this medium with the addition of agar-agar or with the replacement of saccharose by lactose.

By a method which was developed at the Scientific-research Institute of Epidemiology and Hygiene a live dry plague vaccine was obtained from the EV strain. It was characterized by a high degree of immunogenicity and a fully satisfactory degree of stability: at 4-8° the dry vaccine preserves its activity for a year and more (Faybich, 1947; Faybich and Karneyev, 1947). This method (with a refinement of technology and equipment) is used up to the present time in the USSR for the production of live dry plague vaccine. Based on a review by Girard (1963) the problem of obtaining a stable live dry plague vaccine has still not been solved. The vaccine is stabilized by means of storage at -23° or it is used in a 15-day period after it is prepared.

The dry live plague vaccine which was developed in 1940-1942 at the Scientific-research Institute of Epidemiology and Hygiene was the prototype for the creation of a number of other live dry vaccines. A great deal of fruitful work on the improvement of dry vaccine and the development of equipment for this purpose was carried out by Dolinov (1960) and other authors. Using, on the one hand, the experience of the industrial preparation of dry plague vaccine, and on the other - Gayskiy tularemia vaccine strains (and later the new vaccine strains No 10, 33, and 53 NIIEG),

Faybich and Tamarina (1947) developed a method and technology for the production of dry live tularemia vaccine. The authors proposed thick and semiliquid nutrient media for the incubation of bacterial cultures, a drying medium, and a method for determining the concentration of live cells in the preparation (biological titer) which made it possible to measure out the vaccine. Dry live tularemia vaccine was initially applied subcutaneously, but with an accumulation of experience in cutaneous application, which was proposed by Elbert for the liquid preparation, the latter method of application was introduced into practice (Faybich and Tamarina, 1947; Zlatovskiy and associates, 1947).

The obtaining, on an industrial scale, of a sufficiently stable live dry tularemia vaccine, the MIVS, with periods of suitability up to a year made it possible to considerably expand the sphere of its application. This prophylactic preparation played an important role in combatting tularemia in the USSR, especially in the first postwar years when the mass multiplication of rodents was observed on territory which was occupied earlier. This created the threat of contaminating the population with tularemia in many regions of the country.

As a result of the lengthy and extensive use of the live tularemia vaccine, the harmlessness of this preparation and its high degree of reactogenicity during cutaneous application have been proven. A single vaccination protects the inoculated persons from the disease during any method of contamination - through the skin, respiratory organs, through the digestive tract, and by other routes. The duration of postvaccination immunity reaches 4-5 years. Under the conditions of an epidemic outbreak of tularemia by means of vaccination it is possible to liquidate it in 10-15 days. In regions which are unsafe for tularemia, with an almost general immunization of the population (90% and more) morbidity can be prevented. Numerous observations on the effectiveness of live tularemia vaccine were generalized by Olsufyev (1953) - a leader and participant in the majority of these works.

Dry live tularemia vaccine is exported from the USSR to other countries. A thorough study of this preparation was conducted in the USA, where it received a high evaluation. This demonstrated the doubtless advantage of this preparation over all known tularemia vaccines made from killed microbes and the antigens extracted from them (Woodward, 1961).

The section concerning the live tularemia vaccine, which is a great achievement of Soviet microbiologists and immunologists, may be summarized by the words of Pavlovskiy, who wrote that the results of the application of this preparation prove "the great prophylactic value of the live tularemia vaccine and set it up

on the same level with smallpox vaccine, which up till now is considered the best of all known vaccines."

The development of methods for the industrial preparation of dry live vaccines against tularemia and plague have considerably facilitated the obtaining of live vaccines against other infections.

Extensive investigations on the immunity and immunoprophylaxis of brucellosis, which have been carried out for many years by Zdrodovskiy and associates (1953), led the authors to the conclusion of the doubtless advantage of live vaccines. After studying a number of Soviet and foreign strains of Brucella with attenuated virulence the selection of the authors fell on strain No 19 of the bovine type of Brucella, which was obtained in the USA in 1923. This strain is recognized as the vaccine strain for the active immunization of farm animals. But the feasibility of using it for immunizing man against brucellosis was demonstrated and put into practice only by Soviet investigators.

From a culture of strain No 19, obtained from the USA, Vershilova selected a stable harmless and immunogenic variant. It was designated No 19-BA. Under the guidance of Zdrodovskiy and taking into consideration the experience of obtaining other live dry vaccines in the USSR, Vershilova and associates at the Gamaleya Institute of Epidemiology and Microbiology developed the technology for the mass production of live dry vaccine from the vaccine strain No 19-BA. In extensive experiments of vaccination of humans the dose of vaccine for a single subcutaneous application was established and the immunological and epidemiological effectiveness of vaccination was studied (Zdrodovskiy, 1953; Vershilova, 1964).

Simultaneously with the first tests of the brucellosis live vaccine in experiments on the immunization of people tests were also made of a brucellosis live dry vaccine which was obtained from strain No 19. In large contingents of people the practical parity of subcutaneous and cutaneous vaccination of people with this vaccine was shown (Faybich, 1947; Karakulov, 1956).

Beginning in 1953-1954 in the USSR the planned vaccination with live dry vaccine was begun on persons who were subjected to the danger of brucellosis infection, mainly workers on farms and in certain other professional groups of the population. The circumstance that the activity of dry vaccine during expedient storage does not change for a year makes it possible to transport and use it in any area of the country.

During primary vaccination of persons who previously have not come in contact with brucellosis infection, the vaccine manifests itself as a weakly reactogenic preparation. But in connection with mass vaccination of the population in areas and regions which are unsafe in respect to brucellosis, in persons who had been sensitized to the brucellosis antigen there often develops a strong postvaccination reaction of an allergic nature. Similar reactions were also observed during the subcutaneous revaccination with brucellosis vaccine.

For the purpose of lowering the reactogenicity of the brucellosis vaccine, initially during revaccination, and beginning with 1957 also during primary vaccinations of sensitized persons, the cutaneous, scarification method began to be used. The adequate effectiveness of this method of inoculation, which was approved earlier for the administration of the live brucellosis M-10 vaccine, also found confirmation in the use of the vaccine from the Gamaleya Institute of Epidemiology and Microbiology. Cutaneous vaccination excludes the possibility of strong reactions of an allergic nature in persons who are sensitized to the brucellosis antigen, and in connection with this the necessity is eliminated for the preliminary general investigation of those being inoculated for the presence of sensitization. This in its turn facilitates the mass vaccination coverage of the population or groups of people who are liable to specific immunization against brucellosis.

In experiments on animals and in epidemiological observations it was established that vaccine from strain No 19 or No 19-BA protects to an equal degree from infection by Brucella of the bovine and goat-sheep type, which is particularly pathogenic for man. Although active immunization of man cannot be considered the main anti-epidemic measure for brucellosis, all the same vaccination, based on the combined data of Zdrodovskiy (1953) and Vershilova (1964), is capable of reducing human morbidity during an unfavorable epidemiological situation by 5-10 times. Along with other anti-epidemic measures, vaccination of farm animals in particular, immunization of man with live brucellosis vaccine which was developed by Soviet investigators plays a significant role in the complex of measures directed at the sharp reduction of brucellosis incidence in the USSR.

After the first investigations in 1925-1926, active immunization against tuberculosis with the live BCG vaccine received all the more recognition and dissemination in the USSR. Already in 1930-1931 the effect of vaccination was noted in the lowering of incidence and morbidity from tuberculosis in children in the first

year of life. In 1937-1942 the transition from vaccination of newborn in families which were unsafe in respect to tuberculosis to the vaccination of all the newborn was carried out. During this period the liquid enteral BCG vaccine was also used. The instability of this preparation, limiting the period for using it to two weeks after preparation, did not permit an expansion of the mass vaccine prophylaxis of tuberculosis. Therefore it was necessary to develop a more stable - dry - preparation. The first preparation, dried in a solution of glucose as a stabilizer, was proposed by Leshchinskaya (1946). It turned out to be much more stable than the liquid BCG vaccine. But all the same during the process of preparing the vaccine and drying it a large number of cells of the vaccine strain died off. This led to a lowering of immunogenicity. Better results were obtained during transition to the production of a dry saccharose-agar-gelatin BCG vaccine. The method for preparing this was proposed by Kozlov and Chalisov (1948). In 1949-1953 the dry saccharose--agar-gelatin BCG vaccine partially, and after 1953 completely, replaced the liquid vaccine. This made it possible to expand immunized contingents and bring the use of the dry BCG vaccine to any region of the country. At the present time in the USSR it is compulsory that all newborn children who do not have contraindications be inoculated with the live BCG vaccine. This is followed by periodic revaccinations up to the 30th year. Active immunization with live vaccine ensures a sharp reduction of tuberculosis incidence among young children. Tuberculosis incidence in vaccinated persons proceeds in a more favorable form.

After the work of Soviet authors, who had obtained a sufficiently stable dry preparation of BCG vaccine, this method attracted the attention of scientists in France, the USA, England, and Japan. At the present time in these countries they also use the dry BCG vaccine. Soviet microbiologists in their turn made use of the experience of foreign authors and at the present time in the USSR a BCG vaccine is being produced which is dried in a solution of sodium glutaminate, thus increasing the thermostability of the preparation. Besides this the vaccine is administered by the intracutaneous method, which turns out to be more effective. The number of single and repeated immunizations with BCG vaccine in the USSR reaches 6 million persons a year (Togunova, 1960).

In 1965 in the Soviet Union production in a dry form was begun of the live STI anthrax vaccine in the form of an improved concentrated preparation. The dry concentrated STI vaccine, according to preliminary data, possesses a high degree of immunological effectiveness and can be preserved for a long time.

Investigations for the purpose of obtaining live vaccines

enriched our knowledge in the area of the biology of causative agents of infections and made a large contribution to theoretical immunology. One cannot forget the thorough study of a unique form of mutability of pathogenic bacteria, leading to the formation of useful ("tamed") variants which are used as vaccine strains. In particular these investigations showed that it is considerably easier to obtain nonpathogenic immunogenic forms of microbes than to stabilize these properties, to preserve the practical usefulness of these forms for a prolonged period. In connection with this a number of methods have been developed for stabilizing the immunogenic properties of vaccine strains. These are based, first of all, on their storage in the form of lyophilized dry cultures and, secondly, on the application of the principle of "animalization," which was already proposed by Tsenkovskiy (Pokrovskaya, 1934; Elbert and Gayskiy, 1945; Chalisov and Tamarina, 1946; Ginsburg, 1946; Korobkova, 1956; Zhukov-Verezhnikov and Sokolov, 1960; Yemelyanova, 1963; Vershilova, 1964; Nikolayev, 1964; Saltykov, 1965).

Wide approval has been received by the specific, for the majority of live vaccines, cutaneous (scarification) method for the immunization of man. It is effective, simple, and safer than subcutaneous injection. There is great interest in the inhalation method of immunization which is being developed in the USSR (Pokrovskaya, 1934; Aleksandrov and associates, 1961; and others). It may turn out to be useful in the event of the necessity for the rapid mass immunization against certain infections - plague, anthrax.

Regardless of the opinion which existed earlier that associated inoculations are possible only with killed and "chemical" vaccines, the numerous investigations of Soviet authors have demonstrated the feasibility of successful associated immunization with live vaccines not only in experiments, but also in anti-epidemic practice.

For the purpose of standardizing live bacterial vaccines, in the USSR recently a regulation has been established according to which for the preparation of live vaccines common vaccine strains are used throughout the entire country. These are systematically controlled and issued as "reference" strains by the Control Institute for Medicinal Biological Preparations imeni Tarasevicha (Saltykov, 1963).

In the development of the problem of live vaccines both in a theoretical and in a practical respect the Soviet Union occupies one of the leading positions in the world. Before us stand great tasks for the further development of this problem. In our opinion

the most important tasks facing our investigators can be formulated in the following manner: obtaining new stable highly immunogenic vaccine strains of bacteria on the basis of methods of contemporary genetics; a more thorough study of the regularities of immunogenesis during the use of live vaccines; improving the technology of production and the equipment for creating standard conditions for obtaining live vaccines - one of the resolving factors for increasing the quality of these preparations.

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